REMARKS

Claims 1-20 are pending with claims 6, 9 and 15-17 withdrawn from consideration as being directed to a non-elected species. Accordingly, claims 1-5, 7-8, 10-14 and 18-20 are under examination. Claims 1 and 19 have been amended. New claims 21 and 22 have been added. Support for the amendments and new claims can be found throughout the application as filed. In particular, support for the amendment to claim 1 can be found, for example, in original claim 19 and page 3, lines 3-5, page 4, lines 25-27, page 5, lines 21-25, and page 12, lines 15-22. Support for new claims 21 and 22 can be found, for example, in original claims 1 and 19. Accordingly, the amendments and new claims do not raise an issue of new matter and entry thereof is respectfully requested. Applicants have reviewed the Office Action mailed February 8, 2008, and respectfully traverse all grounds of rejection for the reasons that follow.

Applicants appreciate the acknowledgement of the priority claim to provisional application 60/395,763, filed July 10, 2002.

Rejections Under 35 U.S.C. § 101

Claims 1-5, 7, 8, 10-14 and 18-20 stand rejected under 35 U.S.C. § 101 as allegedly being directed to a non-statutory process. Applicants respectfully submit that the claimed methods are directed to a statutory process. Nevertheless, to further prosecution, claim 1 has been amended to recite "providing an output of the at least one candidate gene deletion to a user." Claim 19 has been amended to recite "outputting the at least one candidate gene deletion to a user." Applicants appreciate the suggestion by Examiner Skowronek of amendments that could overcome the rejection. Applicants respectfully submit that this rejection has been rendered moot and request that this rejection be withdrawn.

Rejections Under 35 U.S.C. § 103

Claims 1, 5, 7, 8, 10, 11, 13, 14, 19 and 20 stand rejected under 35 U.S.C. § 103 as allegedly obvious over Hatzimanikatis et al., *AIChE J.* 42:1277-1292 (1996), in view of Bhaskar et al., *Rev. Chem. Eng.* 16:1-54 (2000), and in view of Anandalingam et al., *Annals Operations Res.* 34:1-11 (1992). Applicants respectfully submit that the claimed methods are unobvious over Hatzimanikatis et al., alone or in combination with Bhaskar et al. and/or Anandalingam et al.

Claim 1, as amended is directed to a method for determining candidates for gene deletions using a model of a metabolic network associated with an organism, the model

comprising a plurality of metabolic reactions defining metabolite relationships, by selecting at least one bioengineering objective function for the organism; selecting at least one cellular objective function; forming a linear optimization problem that couples the at least one cellular objective function with the at least one bioengineering objective function; solving the linear optimization problem to yield at least one candidate gene deletion; and providing an output of the at least one candidate gene deletion to a user. Claim 19, as amended, is directed to a computer-based method for determining candidates for gene deletions using a model of a metabolic network associated with an organism, the model comprising a plurality of metabolic reactions defining metabolite relationships, by inputting at least one bioengineering objective function; receiving as input as least one cellular objective function; forming a linear optimization problem that quantifies the at least one cellular objective function as an aggregate reaction flux and couples the at least one cellular objective function with the at least one bioengineering objective function; solving the linear optimization problem to yield at least one candidate gene deletion; and outputting the at least one candidate gene deletion to a user.

Applicants respectfully submit that Hatzimanikatis et al., alone or in combination with Bhaskar et al. and/or Anandalingam et al., does not teach or suggest the claimed methods for determining candidates for gene deletions. With respect to Hatzimanikatis et al., Applicants respectfully submit that this reference, at best, describes design of metabolic reaction networks through modification of "regulatory structures." Hatzimanikatis et al. describes:

> Prior research and industrial practice have clearly shown that very large increases in process performance can be realized by genetic modification of metabolic control systems... Modifying the regulatory characteristics of an enzyme is presently a much more difficult experimental challenge than changing the amount of enzyme present in a cell. Therefore, guidance as to what changes in regulation might be of greatest benefit to improve the network is important. To this end, a systematic, multilevel, muliparametric methodology for evolving effective control structures is needed. [page 1278, column 1, third paragraph, emphasis added].

> The objective of this work is to provide a mathematical framework for determining changes in regulatory structure and strength which should be considered to optimize a particular metabolic process. [page 1278, column 2, third complete paragraph, emphasis added]

Hatzimanikatis et al. further describes the nature of the regulatory structure of a metabolic network as follows:

> The regulatory structure of a metabolic network is typically deduced from experimental analysis of the integrated system or from the reported kinetic properties of the enzymes involved in the pathway. In this case the matrix ε can be written as a sum of two matrices
>
> $$\varepsilon = \varepsilon^s + \varepsilon^r$$
>
> where the elements in the matrix ε^s correspond to the substrate elasticities of the enzymes, that is the sensitivities of the enzyme activities with respect to their substrates, and the elements of matrix ε^r correspond to the regulatory elasticities of the enzymes, that is, the sensitivities of enzyme activities with respect to regulatory metabolites. [page 1280, paragraph bridging columns 1 and 2]

In one example described in Hatzimanikatis et al., the reference states that "[T]herefore, the solution suggests that the enzymes that catalyze reactions 5 and 6 in the pathway should be engineered, if possible, so that both will be inhibited by either CHR [chorismate] and PHE [phenylalanine]" (page 1286, right column). Thus, Hatzimanikatis et al., at best, describes modification of regulatory structures of metabolic pathways. However, Hatzimanikatis et al. provides no teaching or suggestion of a method for determining candidates for gene deletions by forming a linear optimization problem that couples at least one cellular objective function with at least one bioengineering function and solving the linear optimization problem to yield at least one candidate gene deletion. Applicants note the acknowledgement in the Office Action on page 7 that "Hatzimanikatis et al. does not show the cellular and bioengineering objective functions that are coupled in a single optimization problem."

The Office Action states on page 6 that:

> Hatzimanikatis et al. shows a bioengineering objective function in eqn. 32 relating to the production of phenylalanine (p. 1284, col. 1). Hatzimanikatis et al. suggests that cellular growth rate can be defined as an objective function (p. 1278, col. 1).

Referring to the acknowledgement in the Office Action that Hatzimanikatis et al. does not show the cellular and bioengineering objective functions that are coupled in a single optimization problem, Applicants further submit that Hatzimanikatis et al. teaches away from the claimed methods of forming a linear optimization problem that couples at least one cellular objective function with at least one bioengineering objective function. In the context of growth rates as an "objective function," Hatzimanikatis et al. states:

> While explicit formulation of the objective function for natural metabolism is nontrivial, prior investigators have proposed maximization of growth rates or most efficient utilization of cellular energetic and chemical resources as the objective function for evolution of natural metabolism [citations omitted]. However, in chemical and pharmaceutical manufacturing that utilize cultivated microorganisms, it is desirable to identify **a different configuration of fluxes** which directs raw materials to products efficiently at high rates and in the presence of high concentrations of product. A production-oriented evolution is needed to achieve these goals. [page 1278, column 1, first paragraph, emphasis added]

Thus, Hatzimanikatis et al. clearly describes and emphasizes that the use of cultivated microorganisms for chemical or pharmaceutical manufacturing requires different considerations from the evolutionary pressure of maximization of growth rates in "natural metabolism." Accordingly, Applicants respectfully submit that Hatzimanikatis et al. teaches away from the claimed methods, in which a linear optimization problem is formed that couples at least one cellular objective function with at least one bioengineering objective function and solves the linear optimization problem to yield at least one candidate gene deletion.

With respect to Bhaskar et al., this reference, at best, describes multiobjective optimization. Accordingly, Applicants respectfully disagree with the assertion in the Office Action on page 8 that it would have been obvious to "modify the linear programming and objective functions to predict metabolic pathway alterations of Hatzimanikatis et al. with the multiobjective optimization and dual/primal optimization problems of Bhaskar et al." To the contrary, one skilled in the art would not have been motivated to modify the teachings of Hatzimanikatis et al. to apply multiobjective optimization as described in Bhaskar et al. because Hatzimanikatis et al. teaches that a "different configuration of fluxes" is required for utilizing cultivated microorganisms for manufacturing a chemical or pharmaceutical versus maximization of growth rates as "an objective function for evolution of natural metabolism." Thus, Applicants submit that Hatzimanikatis et al., alone or in combination with Bhaskar et al., provides no teaching or suggestion of the claimed methods, in which a linear optimization problem is formed that couples at least one cellular objective function with at least one bioengineering objective function and is solved to yield at least one candidate gene deletion.

With respect to Anandalingam et al., the only comments in the Office Action on this reference are as follows (see page 7, last paragraph):

> Anandalingam et al. shows the bilevel optimization problems described in Bhaskar et al. Anandalingam et al. shows that the decisions made by one agent, an objective function and a set of decision variables, affects the decisions made by the other agents (abstract).

Applicants respectfully submit that Anandalingam et al., at best, describes hierarchical optimization but provides no teaching or suggestion that can be considered to render the claimed methods obvious. Applicants respectfully submit that the Office Action does not articulate how Anandalingam et al. is considered to render the claims obvious in combination with Hatzimanikatis et al. and/or Bhaskar et al. As stated in MPEP § 2141 (II):

> Once the findings of fact are articulated, Office personnel must provide an explanation to support an obviousness rejection under 35 U.S.C. 103. 35 U.S.C. 132 requires that the applicant be notified of the reasons for the rejection of the claim so that he or she can decide how best to proceed. Clearly setting forth findings of fact and the rationale(s) to support a rejection in an Office action leads to the prompt resolution of issues pertinent to patentability.

For the reasons discussed above, Applicants submit that the claimed methods are unobvious over Hatzimanikatis et al., alone or in combination with Bhaskar et al. Nevertheless, if the rejection is maintained, Applicants respectfully request that the reasons for rejection of the claims with respect to Anandalingam et al. be provided so that any issues pertinent to patentability in view of this reference can be resolved.

As discussed above, Applicants respectfully submit that the claimed methods are unobvious over Hatzimanikatis et al., alone or in combination with Bhaskar et al. and/or Anandalingam et al. Accordingly, Applicants respectfully request that this rejection be withdrawn.

Claims 1, 2, 4 and 18 stand rejected under 35 U.S.C. § 103 as allegedly obvious over Hatzimanikatis et al., *supra*, in view of Bhaskar et al., *supra*, and in view of Anandalingam et al., *supra*, and further in view of Yang et al., *Metabolic Engineering* 1:26-34 (1999). Applicants respectfully submit that the claimed methods are unobvious over Hatzimanikatis et al., alone or in combination with Bhaskar et al. and/or Anandalingam et al. and/or Yang et al.

As discussed above, Applicants respectfully submit that Hatzimanikatis et al., alone or in combination with Bhaskar et al. and/or Anandalingam et al., does not teach or suggest the claimed methods. Furthermore, Applicants respectfully submit that Yang et al. does not cure the deficiencies of Hatzimanikatis et al., alone or in combination with Bhaskar et al. and/or

Anandalingam et al. Yang et al., at best, describes the use of metabolic flux analysis in an *Escherichia coli* strain deficient in the acetate production pathway. However, as discussed previously on the record, Yang et al. does not discuss forming an optimization problem that couples at least one cellular objective function with a bioengineering objective function. Accordingly, Applicants respectfully submit that the claimed methods are unobvious over Hatzimanikatis et al., alone or in combination with Bhaskar et al. and/or Anandalingam et al. and/or Yang et al. Therefore, Applicants respectfully request that this rejection be withdrawn.

Claims 1, 5, 7, 8, 10-14, 19 and 20 stand rejected under 35 U.S.C. § 103 as allegedly obvious over Burgard et al., *Biotechnol. Bioeng.* 74:364-375 (2001), in view of Bhaskar et al., *supra*, and in view of Anandalingam et al., *supra*. Applicants respectfully submit that the claimed methods are unobvious over Burgard et al., alone or in combination with Bhaskar et al. and/or Anandalingam et al.

Applicants respectfully maintain, for the reasons of record, that Burgard et al. does not teach or suggest the claimed methods of determining candidates for gene deletions by forming a linear optimization problem that couples at least one cellular objective function with at least one bioengineering objective function and solving the linear optimization problem to yield at least one candidate gene deletion. As discussed previously on the record, Burgard et al. discusses a gene knockout study that explores what is the smallest gene set capable of maximizing biomass production on glucose substrate and what is the maximum number of gene deletions from this set that still maintain a specified level of biomass production. Separately, Burgard et al. discusses identifying mathematically optimal reaction pathways to recombine into the *E. coli* metabolic network to optimize amino acid formation for growth on glucose and acetate. However, as discussed previously on the record, Burgard et al. provides no teaching or suggestion of forming and solving an optimization problem that couples a bioengineering objective function with a cellular objective function.

It is clear from the description in Burgard et al. that the determination of the smallest gene set capable of maximizing a cellular function such as biomass production is distinct and separate from identifying optimal reaction pathways to optimize a bioengineering objective such as optimization of amino acid formation. In particular, Burgard et al. discloses that "[I]t is the objective of this study to utilize FBA and mixed-integer programming tools to select the mathematically optimal genes for recombination into *E. coli* from a metabolic database

encompassing many genes from multiple species" (page 365, column 2, paragraph bridging columns 1 and 2). As a separate goal of the modeling studies, Burgard et al. describes the desire of determining the smallest number of genes required for growth. Burgard et al. states that the "recent upsurge of sequenced genomes has also brought significant attention to the question of which genes are crucial for supporting cellular life... Determining the maximum number of tolerable gene deletions in a given metabolic system, however, requires a discrete optimization strategy in which multiple gene deletions can be simultaneously examined." (page 365, right column, first complete paragraph; emphasis added). Burgard et al. concludes from their studies that "[T]he proposed optimization framework provided the quantitative means to study metabolic network performance limits in response to gene deletions or additions. Metabolic network performance relates to either robustness in the face of gene deletions or flux enhancements through foreign gene recombination from an ever-expanding database of available genes" (page 373, right column, first paragraph of "Conclusions;" emphasis added). Thus, throughout Burgard et al., the goal of determining multiple gene deletions to maintain cell growth or the goal of determining gene additions to produce a desired product are considered separate goals that utilize similar mathematical modeling (see page 367, right column, "[T]he selection of the optimal gene choices for deletion or insertion from DNA recombination can be determined by appropriately constraining the number of nonzero elements in y," emphasis added). However, no where does Burgard et al. teach or suggest any desirability of forming a linear optimization problem that couples a cellular objective function with a bioengineering objective function and solving to determine at least one candidate gene deletion.

The Office Action acknowledges on page 12 that "Burgard et al. do not teach the generation of a bilevel optimization problem or the coupling of cellular and bioengineering objective functions." Furthermore, as discussed above, Burgard et al. describes the determination of a set of gene deletions that provides the smallest gene set to achieve a desired level of growth and the determination of gene additions to produce a desired product as separate goals achieved using mathematical calculations for optimizing each goal. However, Applicants respectfully disagree with the assertion in the Office Action on page 13 that it would have been obvious to one skilled in the art to modify the linear programming and objective functions to predict metabolic pathways of Burgard et al. with the multiobjective optimization and dual/primal optimization problems of Bhaskar et al. The fact that Burgard et al. separately

describes the goal of optimizing a bioengineering objective by determining gene additions or optimizing a cellular objective by determining the smallest gene set that permits the cell to attain specified levels of biomass production in no way provides one skilled in the art with the desirability of modifying the description in Burgard et al. with the multiobjective optimization of Bhaskar et al. to form a linear optimization problem that couples a cellular objective function with a bioengieneering function and to solve the linear optimization problem to yield at least one candidate gene deletion.

With respect to Anandalingam et al., as discussed above, Applicants respectfully submit that this reference, at best, describes hierarchical optimization but provides no teaching or suggestion that can be considered to render the claimed methods obvious. Furthermore, Applicants respectfully submit that the Office Action does not articulate how Anandalingam et al. is considered to render the claims obvious in combination with Burgard et al. and/or Bhaskar et al. For the reasons discussed above, Applicants submit that the claimed methods are unobvious over Burgard et al., alone or in combination with Bhaskar et al. Nevertheless, if the rejection is maintained, Applicants respectfully request that the reasons for rejection of the claims with respect to Anandalingam et al. be provided so that any issues pertinent to patentability in view of this reference can be resolved.

As discussed above, Applicants respectfully submit that the claimed methods are unobvious over Burgard et al., alone or in combination with Bhaskar et al. and/or Anandalingam et al. Accordingly, Applicants respectfully request that this rejection be withdrawn.

Claims 1-4 and 18 stand rejected under 35 U.S.C. § 103 as allegedly obvious over Burgard et al., *supra*, in view of Bhaskar et al., *supra*, and in view of Anandalingam et al., *supra*, and further in view of Yang et al., *supra*. Applicants respectfully submit that the claimed methods are unobvious over Burgard et al., alone or in combination with Bhaskar et al. and/or Anandalingam et al. and/or Yang et al.

As discussed above, Applicants respectfully submit that Burgard et al., alone or in combination with Bhaskar et al. and/or Anandalingam et al., does not teach or suggest the claimed methods. Furthermore, Applicants respectfully submit that the Yang et al. does not cure the deficiencies of Burgard et al., alone or in combination with Bhaskar et al. and/or Anandalingam et al. Yang et al., at best, describes the use of metabolic flux analysis in an *E. coli* strain deficient in the acetate production pathway. However, as discussed previously on the

record, Yang et al. does not discuss forming an optimization problem that couples at least one cellular objective function with a bioengineering objective function. Accordingly, Applicants respectfully submit that the claimed methods are unobvious over Burgard et al., alone or in combination with Bhaskar et al. and/or Anandalingam et al. and/or Yang et al. Therefore, Applicants respectfully request that this rejection be withdrawn.

CONCLUSION

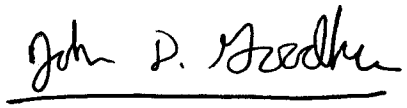
In light of the Amendments and Remarks herein, Applicant submits that the claims are in condition for allowance and respectfully request a notice to this effect. Should the Examiner have any questions, he is invited to call the undersigned attorney.

This is a request under the provision of 37 CFR § 1.136(a) to extend the period for filing a response in the above-identified application for three months from May 8, 2008 to August 8, 2008. Applicant is a small entity; therefore, please charge Deposit Account No. 26-0084 in the amount of $525.00 to cover the cost of the three-month extension. Any deficiency or overpayment should be charged or credited to Deposit Account 26-0084.

No other fees or extensions of time are believed to be due in connection with this amendment; however, consider this a request for any extension inadvertently omitted, and charge any additional fees to Deposit Account No. 26-0084.

Reconsideration and allowance is respectfully requested.

Respectfully submitted,



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